LABORATORY

4

Chemical Composition of Cells

Learning Outcomes

Introduction
- Describe how cells build and break down polymers.

4.1 Proteins
- State the function of various types of proteins.
- Describe a test for the detection of a protein versus a peptide.

Prelab Question: When testing a food for protein, what test might you use and how would you do the test?

4.2 Carbohydrates
- Identify the subunit of starch, and distinguish sugars from starch in terms of subunit number.
- Describe a test for the detection of starch and another for the detection of sugars.

Prelab Question: Why is it a good idea to avoid foods high in sugars?

4.3 Lipids
- Name several types of lipids, and state their components.
- Describe a simple test for the detection of fat, and tell how an emulsifier works.

Prelab Question: Why is butter solid at room temperature, while an oil is liquid even when placed in the refrigerator?

4.4 Testing Foods and Unknowns
- Explain a procedure for testing the same food for all three components—carbohydrates, protein, and fat.

Prelab Question: Explain why tests for proteins, carbohydrates, and lipids need to be performed separately.

***Application for Daily Living:* Nutrition Labels**

Introduction

Planning Ahead To save time, your instructor may have you start the boiling water bath needed for the experiments to test for proteins and carbohydrates.

You may be familiar with the terms *carbohydrate, protein, fat,* and *oil* because they are found in the food you eat. In the diet, they are known as nutrients, but in cell biology they are called biomolecules. In this lab, you will be studying biomolecules found in cells: **proteins, carbohydrates,** and **lipids** (fats and oils).

Macromolecules are large biomolecules composed of smaller subunits. Carbohydrates and proteins are macromolecules that are also **polymers** because they are made up of many repeating, smaller subunits called **monomers.** Proteins are composed of amino acid monomers joined together by peptide bonds. Polysaccharides are carbohydrates that consist of long chains of individual monosaccharide monomers joined together. Lipids are macromolecules but are not considered polymers because they contain more than one type of subunit, such as fatty acid, glycerol, or phosphate.

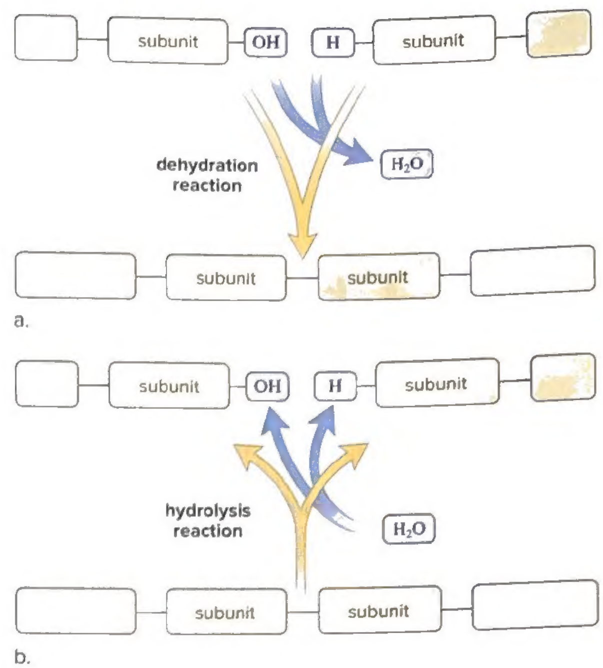
Macromolecules are formed by a **dehydration** reaction, where a water molecule is produced as the smaller subunits are bonded together. In contrast, macromolecules are broken through **hydrolysis** reactions, where a water molecule is added as the bonds between subunits are split.

Testing for Biomolecules

In this lab, you perform tests to investigate which biomolecules are present in unknown substances and food items. Most of the tests involve reagents that will change color and indicate a positive result if the biomolecule is present or a negative result if it is absent.

To ensure the tests work properly, each will include a positive control and a negative control. Both controls go through all steps of the test and serve as a comparison to the unknowns being tested. A **positive control** is a substance known to give a positive result. If an unknown shows the same result as the positive control, you can be sure it contains the biomolecule being tested. The **negative control** is a substance known to give a negative result. If your unknown substance resembles the negative control, the biomolecule is not present.

Positive and negative controls give you a standard by which to tell if the biomolecule being tested is present and to ensure the experiment is giving reliable results. If either the positive or negative control gives unexpected results, then the entire experiment may be faulty.



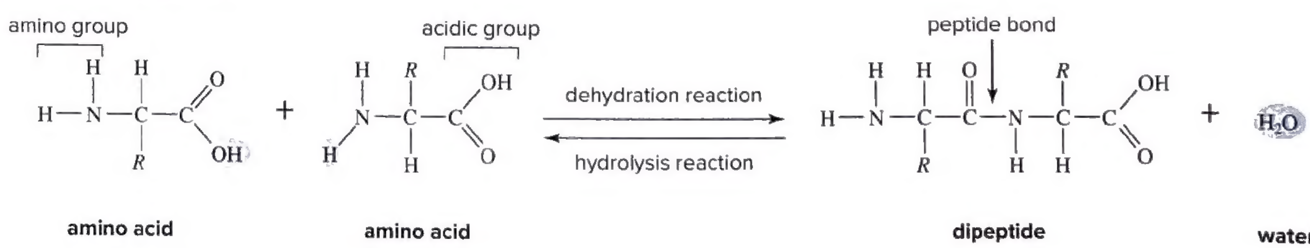
4.1 Proteins

Proteins have numerous functions in living organisms. Antibodies are proteins that combine with pathogens so that the pathogens are destroyed by the body. Transport proteins combine with and move substances from place to place. Hemoglobin transports oxygen throughout the body. Albumin is another transport protein in our blood. Regulatory proteins control cellular metabolism. For example, the hormone insulin regulates the amount of glucose in blood so that cells have a ready supply. Structural proteins include keratin, found in hair, and myosin, found in muscle. **Enzymes** are proteins that speed chemical reactions. A reaction that could take days or weeks to complete can happen within an instant if the correct enzyme is present. Most enzymes end in the letters *-ase*—for example, amyl*ase* is an enzyme that speeds the breakdown of starch in the mouth and small intestine.

Proteins are made up of **amino acids** (the subunits) joined together. About 20 different common amino acids are found in cells. All amino acids have an acidic group (—COOH) and an amino group (H_2N—). They differ by the ***R* group** (remainder group) attached to a carbon atom, as shown in Figure 4.1. The *R* groups have varying sizes, shapes, and chemical activities.

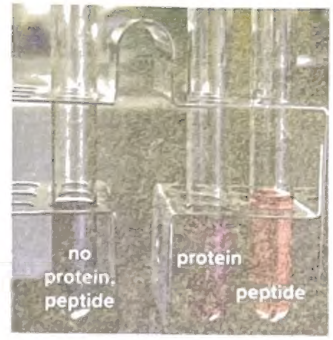
A chain of two or more amino acids is called a **peptide,** and the bond between the amino acids is called a **peptide bond.** A **polypeptide** is a very long chain of amino acids. A protein can contain one or more polypeptide chains. A single chain forms insulin, while four chains form hemoglobin. A protein has a particular shape, which is important to its function. The shape comes about because the *R* groups of the polypeptide chain(s) can interact with one another in various ways.

Figure 4.1 Formation of a dipeptide.
During a dehydration reaction, a dipeptide forms when an amino acid joins with an amino acid as a water molecule is removed. The bond between amino acids is called a peptide bond. During a hydrolysis reaction, water is added, and the peptide bond is broken.



Test for Proteins

Biuret reagent (blue color) contains a strong solution of sodium or potassium hydroxide ($NaOH$ or KOH) and a small amount of dilute copper sulfate ($CuSO_4$) solution. The reagent changes color in the presence of proteins or peptides because the peptide bonds of the protein or peptide chemically combine with the copper ions in biuret reagent (Table 4.1).



Biuret test for protein and peptides

Dave Moyer

Table 4.1 Biuret Test for Protein and Peptides

	Protein	Peptides
Biuret reagent (blue)	Purple	Pinkish-purple

Experimental Procedure: Test for Proteins

1. Label four clean test tubes (1 to 4).
2. Using the designated graduated transfer pipettes, add 1 ml of the experimental solutions listed in Table 4.2 to the test tubes according to their numbers.
3. Then add five drops of biuret reagent to the tubes, swirling to mix.
4. The reaction is almost immediate. Record your observations in Table 4.2.

> **Biuret reagent** Biuret reagent is highly corrosive. Exercise care in using this chemical. If any should spill on your skin, wash the area with mild soap and water. Follow your instructor's directions for its disposal.

Table 4.2 Biuret Test for Protein

Tube	Contents	Final Color	Conclusions (+ or –)
1	Distilled water		
2	Albumin		
3	Pepsin		
4	Starch suspension		

Conclusions: Proteins

- From your test results, conclude if a protein is present (+) or absent (–). Enter your conclusions in Table 4.2.
- According to your results, is starch a protein? ______________________________
- Which of the four tubes is the negative control sample? __________ Why? ______________________________

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4.2 Carbohydrates

Carbohydrates include sugars and molecules that are chains of sugars. **Glucose,** which has only one sugar unit, is a monosaccharide; **maltose,** which has two sugar units, is a disaccharide (Fig. 4.2). Glycogen, starch, and cellulose are polysaccharides, made up of chains of glucose units (Fig. 4.3).

Figure 4.2 Formation of a disaccharide.
During a dehydration reaction, a disaccharide, such as maltose, forms when a glucose joins with a glucose as a water molecule is removed. During a hydrolysis reaction, the components of water are added, and the bond is broken.

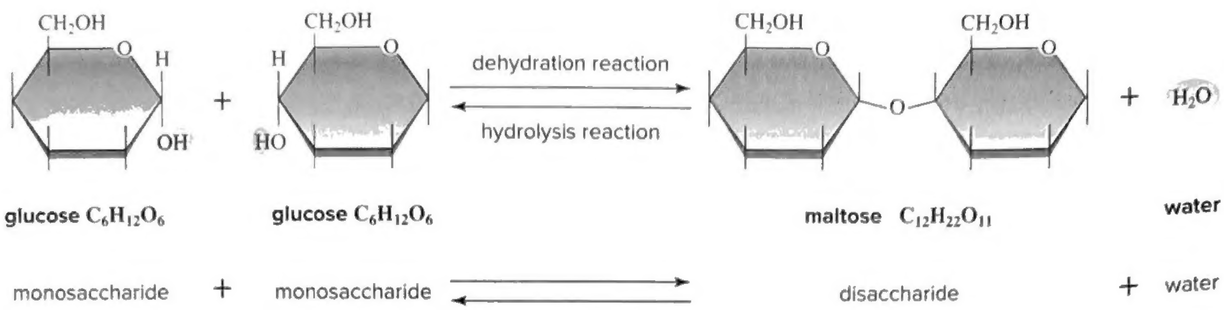
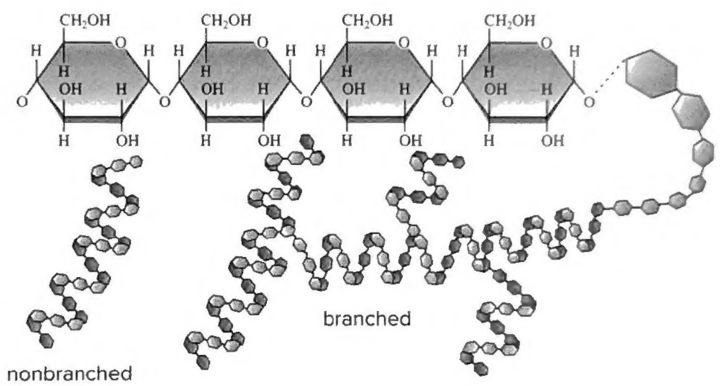


Figure 4.3 Starch.
Starch is a polysaccharide composed of many glucose units. It consists of amylose that is nonbranched and amylopectin that is branched.



Glucose is used by a large number of organisms as an energy source. Energy is released when glucose is broken down to carbon dioxide and water. This energy is used by the organism to do work. Animals store glucose as glycogen, and plants store glucose as starch. Plant cell walls are composed of cellulose.

Test for Starch

In the presence of starch, iodine solution (yellowish-brown) reacts chemically with starch to form a blue-black color (Table 4.3).

Table 4.3 Iodine Test for Starch

	Starch
Iodine solution	Blue-black

Experimental Procedure: Test for Starch

1. Label five clean test tubes (1 to 5).
2. Using the designated graduated transfer pipettes, add 1 ml of the experimental solutions listed in Table 4.4 to the test tubes according to their numbers.
3. Then add five drops of iodine solution to the tubes at the same time.
4. Note the final color changes, and record your observations in Table 4.4.

Table 4.4 Iodine (IKI) Test for Starch

Tube	Contents	Color	Conclusions
1	Distilled water		
2	Glucose solution		
3	Starch suspension		
4	Onion juice		
5	Potato juice		

Conclusions: Starch

- From your test results, draw conclusions about what organic compound is present in each tube. Write these conclusions in Table 4.4.
- Does the potato or the onion store glucose as starch? __________ How do you know? ______________

  ____

Experimental Procedure: Microscopic Study

Potato

1. With a scalpel, slice a very thin piece of potato. Make sure your piece does not contain any potato skin. Place it on a microscope slide, add a drop of water and a coverslip, and observe under low power with your compound light microscope. Find the cell wall (large, geometric compartments) and the starch grains (numerous clear, oval-shaped objects).
2. Without removing the coverslip, place two drops of iodine solution onto the microscope slide so that the iodine touches the coverslip. Suck the iodine under the coverslip by placing a small piece of paper towel in contact with the water on the **opposite** side of the coverslip.
3. Microscopically examine the potato again on the side closest to where the iodine solution was applied. Under a microscope, a positive starch/iodine reaction may appear purple rather than blue-black.

   What is the color of the small, oval bodies? ______________________________

   What is the chemical composition of these oval bodies? ______________________________

Onion

1. Peel a single layer of onion from the bulb. On the inside surface, you will find a thin, transparent layer of onion skin. Peel off a small section of this layer for use on your slide.
2. Add a large drop of iodine solution.
3. Does onion contain starch? ______________________________
4. Are these results consistent with those you recorded for onion juice in Table 4.4? ______________

Test for Sugars

> **Benedict's reagent** Benedict's reagent is highly corrosive. Exercise care in using this chemical. If any should spill on your skin, wash the area with mild soap and water. Follow your instructor's directions for disposal of this chemical.

Monosaccharides and some disaccharides will react with **Benedict's reagent** after being heated in a boiling water bath. In this reaction, copper ion (Cu^{2+}) in the Benedict's reagent reacts with part of the sugar molecule, causing a distinctive color change. The color change can range from green to red, and increasing concentrations of sugar will give a continuum of colored products (Table 4.5).

Table 4.5 Benedict's Test for Sugars (Some Typical Reactions)

Chemical	Chemical Category	Benedict's Reagent (After Heating)	
Water	Inorganic	Blue (no change)	
Glucose Maltose	Monosaccharide (carbohydrate) Disaccharide (carbohydrate)	Varies with concentration:	very low—green low—yellow moderate—yellow-orange high—orange very high—orange-red
Starch	Polysaccharide (carbohydrate)	Blue (no change)	

Experimental Procedure: Test for Sugars

1. Prepare a boiling water bath, and label five clean test tubes (1 to 5).
2. Using the designated graduated transfer pipettes, add 1 ml of the experimental solutions listed in Table 4.6 to the test tubes according to their numbers.
3. Then add five drops of Benedict's reagent to all the tubes at this time.
4. Place the tubes into the boiling water bath at the same time.
5. When, after a few minutes, you see a change of colors, remove all of the tubes from the water bath and record your observations in Table 4.6.
6. Save your tubes for comparison purposes when you do section 4.4.

Table 4.6 Benedict's Test for Sugars

Tube	Contents	Color (After Heating)	Conclusions
1	Distilled water		
2	Glucose solution		
3	Starch suspension		
4	Onion juice		
5	Potato juice		

Conclusions: Sugars

- From your test results, conclude what kind of chemical is present. Enter your conclusions in Table 4.6.
- Which tube served as a negative and which as a positive control? ______________________
- Compare the results in Tables 4.4 and 4.6. In what form are carbohydrates found in onions and potatoes? Explain why. ______________________

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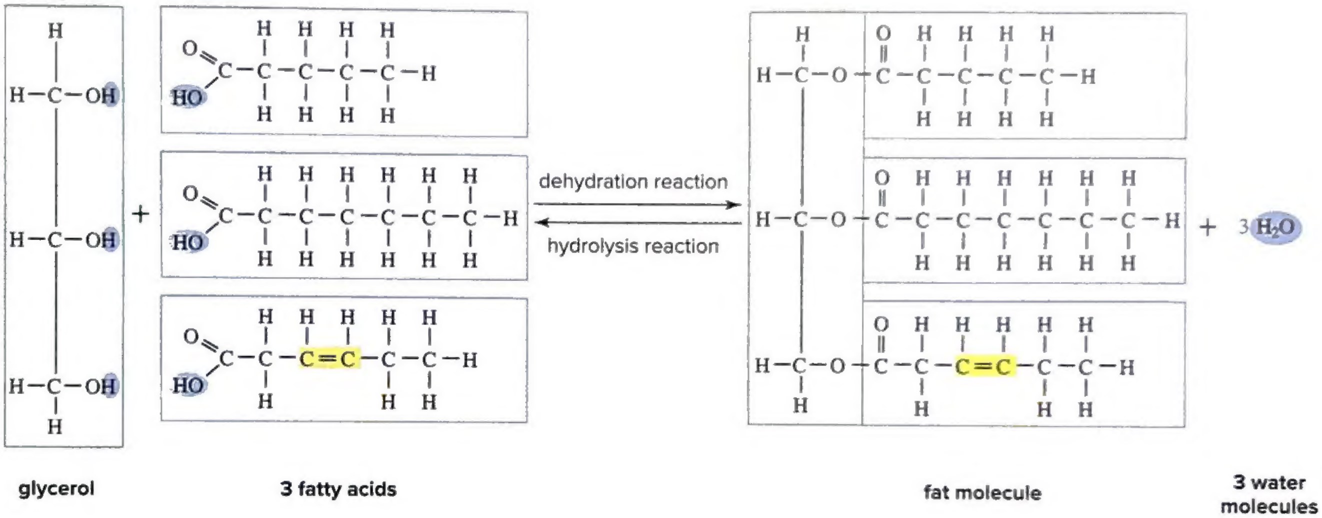
4.3 Lipids

Lipids are compounds that are insoluble in water and soluble in nonpolar solvents, such as alcohol and ether. Lipids include fats, oils, phospholipids, steroids, and cholesterol. Typically, **fat,** such as in the adipose tissue of animals, and **oils,** such as the vegetable oils from plants, are composed of three molecules of fatty acids bonded to one molecule of glycerol (Fig. 4.4). **Phospholipids** have the same structure as fats, except that in place of the third fatty acid there is a phosphate group (a grouping that contains phosphate). **Steroids** are derived from **cholesterol.** These molecules have skeletons of four fused rings of carbon atoms, but they differ by functional groups (attached side chains). Fat, as we know, is long-term stored energy in the human body. Phospholipids are found in the plasma membrane of a cell. Cholesterol, a molecule transported in the blood, has been implicated in causing cardiovascular disease. Regardless, steroids are very important compounds in the body. For example, the sex hormones, such as testosterone and estrogen, are steroids.

Figure 4.4 Formation of a fat.
During a dehydration reaction, a fat molecule forms when glycerol joins with three fatty acids as three water molecules are removed. During a hydrolysis reaction, water is added, and the bonds are broken between glycerol and the three fatty acids.



Test for Lipids

Fats and oils do not evaporate from brown paper or loose-leaf paper; instead, they leave an oily spot.

Experimental Procedure: Paper Test for Fat

1. Place a small drop of distilled water on a square of brown paper or loose-leaf paper. Describe the immediate effect. ______________________________
2. Place a small drop of vegetable oil on a square of the paper. Describe the immediate effect. __________

   ____
3. Wait at least 15 minutes for the paper to dry. Evaluate which substance penetrates the paper and which is subject to evaporation. Record your observations and conclusions in Table 4.7. Save the paper for comparison use with section 4.4.

Table 4.7 Paper Test for Fat		
Sample	**Observations**	**Conclusions**
Water spot		
Oil spot		

Emulsification of Oil

Some molecules are **polar,** meaning that they have charged groups or atoms, and some are **nonpolar,** meaning that they have no charged groups or atoms. A water molecule is polar, and therefore, water is a good solvent for other polar molecules. When the charged ends of water molecules interact with the charged groups of polar molecules, these polar molecules disperse in water.

Water is not a good solvent for nonpolar molecules, such as fats and oils. A lipid has no polar groups to interact with water molecules. An **emulsifier,** however, can cause fats and oils to disperse in water. An emulsifier contains molecules with both polar and nonpolar ends. When the nonpolar ends interact with the fat and the polar ends interact with the water molecules, the fat or oil disperses in water, and an **emulsion** results (Fig. 4.5). Dish soap is an example of an emulsifier. It allows you to remove oil from a pan by dispersing the oil in the dishwater.

Bile salts (emulsifiers found in bile produced by the liver) are used in the digestive tract. Today milk, such as 1% milk, has been homogenized so that fat droplets do not congregate and rise to the top of the container. Homogenization requires the addition of natural emulsifiers, such as phospholipids—the phosphate part of the molecule is polar and the lipid portion is nonpolar.

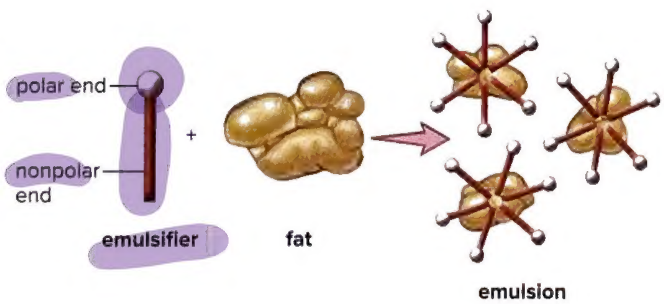


Figure 4.5 Emulsification. An emulsifier contains molecules with both a polar and a nonpolar end. The nonpolar ends are attracted to the nonpolar fat, and the polar ends are attracted to the water. This causes droplets of fat molecules to disperse.

Experimental Procedure: Emulsification of Lipids

Label three clean test tubes (1 to 3), and use the appropriate graduated pipette to add solutions to the test tubes as follows:

Tube 1

1. Add 3 ml of distilled water and 1 ml of vegetable oil. Shake.
2. Is vegetable oil soluble in water? ____________________
3. Let the tube settle for 5 minutes. Label a microscope slide as 1.
4. Use a dropper to remove a sample of the solution that is just below the layer of oil. Place the drop on the slide, add a coverslip, and examine with the low power of your compound light microscope.
5. Record your observations in Table 4.8.

Tube 2

1. Add 3 ml of distilled water, 1 ml of vegetable oil, and 1 ml of the available emulsifier (Tween or bile salts). Shake.
2. Describe how the distribution of oil in tube 2 compares with the distribution in tube 1.

____

____

3. Let the tube settle for 5 minutes. Label a microscope slide as 2.
4. Use a different dropper to remove a sample of the solution that is just below the layer of oil. Place the drop on the slide, add a coverslip, and examine with the low power of your compound light microscope.
5. Record your observations in Table 4.8.

Tube 3

1. Add 1 ml of milk and 2 ml of distilled water. Shake well.
2. Use a different dropper to remove a sample of the solution that is just below the layer of oil. Place the drop on the slide, add a coverslip, and examine with the low power of your compound light microscope.
3. Record your observations in Table 4.8.

Table 4.8 Emulsification

Tube	Contents	Observations	Conclusions
1	Oil Distilled water		
2	Oil Distilled water Emulsifier		
3	Milk Distilled water		

Conclusions: Emulsification

- From your observations, conclude why the contents of each tube appear as they do under the microscope. Record your conclusions in Table 4.8.

4.4 Testing Foods and Unknowns

It is common for us to associate the term *organic* with the foods we eat. Though we may recognize foods as being organic, often we are not aware of what specific types of organic compounds are found in what we eat. In the following Experimental Procedure, you will use the same tests you used previously to determine the composition of everyday foods and unknowns.

Experimental Procedure: Testing Foods and Unknowns

Your instructor will provide you with several everyday foods to test for the presence of protein, carbohydrates, and lipids. Perform each test you learned from the previous exercises, and record your results as positive (+) or negative (–) in Table 4.9.

Table 4.9 Testing Foods and Unknowns

Sample Name	Protein (Biuret)	Starch (Iodine)	Sugar (Benedict's)	Fat (Brown or Loose-Leaf Paper)
Unknown A				
Unknown B				

Application for Daily Living

Nutrition Labels

For good health, the diet should be balanced and should not contain an overabundance of protein, carbohydrate, or fat. Everyone should become familiar with reading nutrition labels. A portion of one is shown in Figure 4.6.

It is very important to note the serving size and number of servings per container. For comparison purposes, you need to compare the same serving sizes. The % Daily Values are based on an intake of 2,000 calories per day. A calorie is an indication of the amount of energy provided, and the number of calories taken in should match the number required per day, unless you wish to gain weight.

Most people are extremely interested in the amount of fat provided by a food. One serving of this food provides 18% of the total fat required for the day. If you find this excessive, you can look for another macaroni and cheese that supplies less total and saturated fat. If you decide that you prefer this product, you might wish to balance it with a food that contains very little fat and one that provides a carbohydrate that contains fiber. This food contains no fiber. Complex carbohydrates containing fiber, such as those found in whole grains, are recommended, and not those that contain simply starch.

Making wise decisions about the foods we buy can lead to a longer, healthier life.

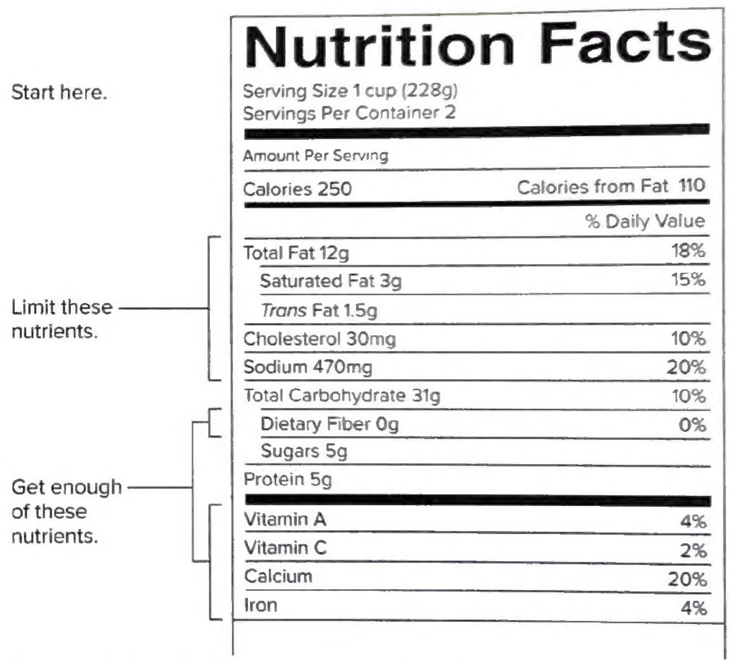


Figure 4.6 Sample nutrition label for macaroni and cheese.
As of January 2006, foods must include the quantity of trans fat. There is no % Daily Value for *trans* fats, and they should be avoided.

Laboratory Review 4

_______________ 1. What term refers to a reaction that adds water to break large biomolecules into subunits?

_______________ 2. What kind of protein is lactase, the biomolecule that speeds up the breakdown of lactose?

_______________ 3. What kind of bond forms during a dehydration synthesis reaction involving two amino acids?

_______________ 4. If maltose undergoes hydrolysis, what subunits result?

_______________ 5. In what form is glucose stored in plants?

_______________ 6. What color does iodine turn if starch is present?

_______________ 7. If a solution contains a large amount of glucose, what color will the Benedict's reagent become?

_______________ 8. What kinds of biomolecules are insoluble in water but soluble in alcohol or ether?

_______________ 9. If two fatty acids, glycerol, and a phosphate group undergo a dehydration reaction, what biomolecule forms?

_______________ 10. What is another name for plant fats?

_______________ 11. What must be present to successfully break down fats during digestion?

_______________ 12. If you test an unknown substance with the biuret reagent and it turns purple, what is present?

_______________ 13. If you test a sample of potato with the biuret reagent, what do you expect the results to be?

_______________ 14. If you want to test a sample for the presence of glucose, what reagent should you use?

Thought Questions

15. What are the similarities between the chemical structure and composition of proteins, carbohydrates, and lipids?

16. After enjoying a meal of fish and chips, you notice your shirt has an oily stain on it. Why won't the oily stain come out when you dab at it with a moist napkin?

17. If you test a solution with biuret reagent and the solution turns purple, what does this indicate? Explain your answer.